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Department of Health and Ageing
Therapeutic Goods Administration

Manufacturing standards for plasma for fractionation

Scientific relevance and regulatory requirements

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Presentation outline

- Current standards
- Empirical observations
- Basic science
- Resulting tensions
- Possible approaches

WARNING :

THIS PRESENTATION IS PEPPERED WITH NOSTALGIA AND OTHER MANIFESTATIONS OF PERSONAL INDULGENCE

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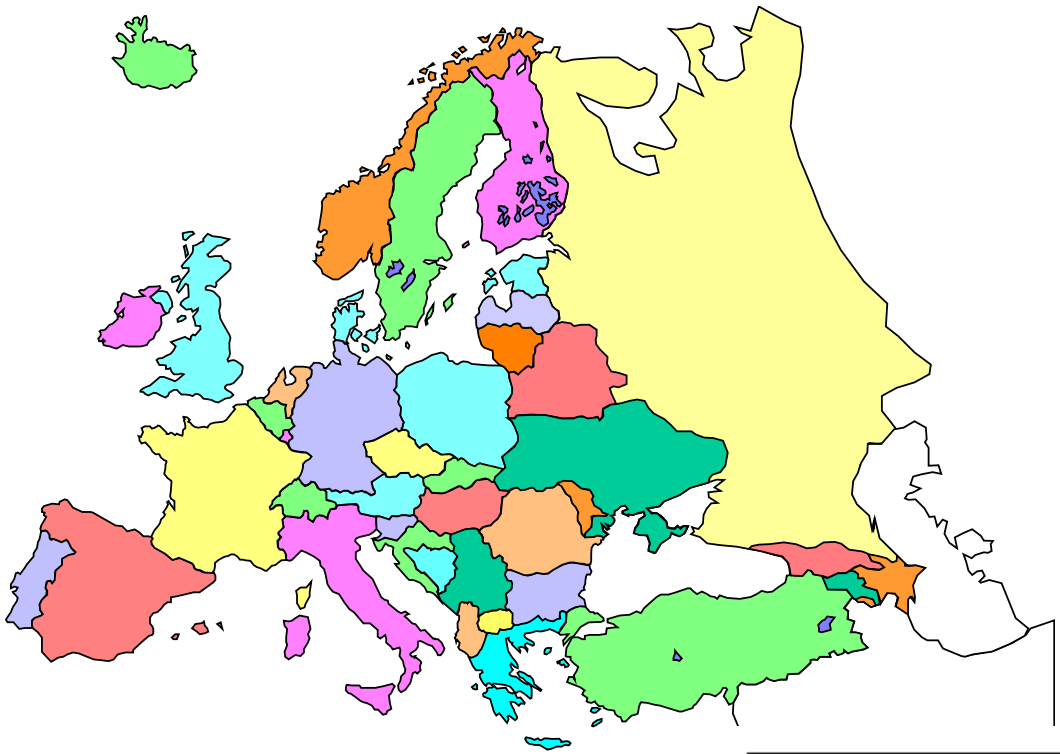
Available standards



2001: 0853

HUMAN PLASMA FOR FRACTIONATION

Plasma humanum ad separationem

Plasma Master File Guideline 2004

2.2.4 Conditions of storage and transport of plasma.

See Annex IV and V.

Describe the conditions for freezing and storage of plasma for every establishment responsible for collecting blood/plasma including the following:

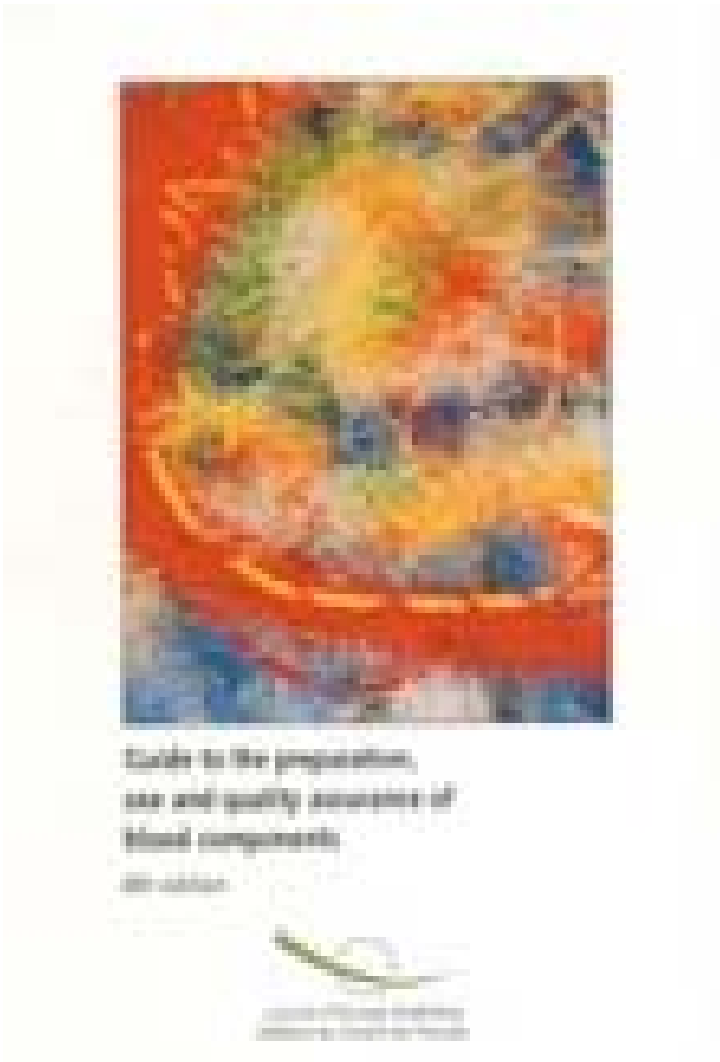
- Sites/organisations which are involved in the storage and indicate whether they have been inspected by a Competent Authority.
- Compliance with Ph. Eur. with respect to freezing and storage.
- Conditions of storage (temperature and maximum time).

Describe the conditions of transport of plasma including the following:

- Transport flows from centres of collection to interim storage sites, if relevant, and further to fractionation sites.
- Organisations which are involved in the transport (own and contractors) and indicate whether they have been inspected by a Competent Authority.
- Conditions of transport (maximum time and temperature).
- Confirm compliance with requirements in the Ph. Eur. Monograph for Human Plasma for Fractionation and if applicable, with any Ph. Eur. requirements for particular products.[9]

[9] Ind

Council of Europe Guide for Blood Components



- Includes chapters on FFP, cryo-poor plasma
- FFP standards sometimes at variance with EP monograph
- NOT APPLICABLE for fractionation - refers to EP monograph

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Available standards

National Archives and Records Administration

code of federal regulations

TITLE 21
FOOD AND DRUGS
PART 640
ADDITIONAL STANDARDS FOR HUMAN BLOOD AND BLOOD PRODUCTS
Subpart G--Source Plasma

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A contentious statement

The regulatory requirements underpinning blood and plasma storage, freezing and frozen storage are predicated on the needs of Factor VIII

FVIII in 2004

- Plasma-derived FVIII production is becoming increasingly marginal in the developed blood economies
- Fractionators still ship plasma for FVIII manufacture in the hope of supplying the “emerging” markets
- Factor VIII is the most labile plasma therapeutic protein
- Conditions affecting FVIII may affect other proteins in ways which are still unknown
- Tailoring conditions to optimising FVIII preservation is therefore still a valid goal

European plasma standards
FVIII levels

Council of Europe

(for transfusion)

Requirement for ≥ 70% of the "*average normal value*" controlled through measurement of FVIIIc every two months on a pool of six units of mixed blood groups during the first and last months of storage

European Pharmacopeia

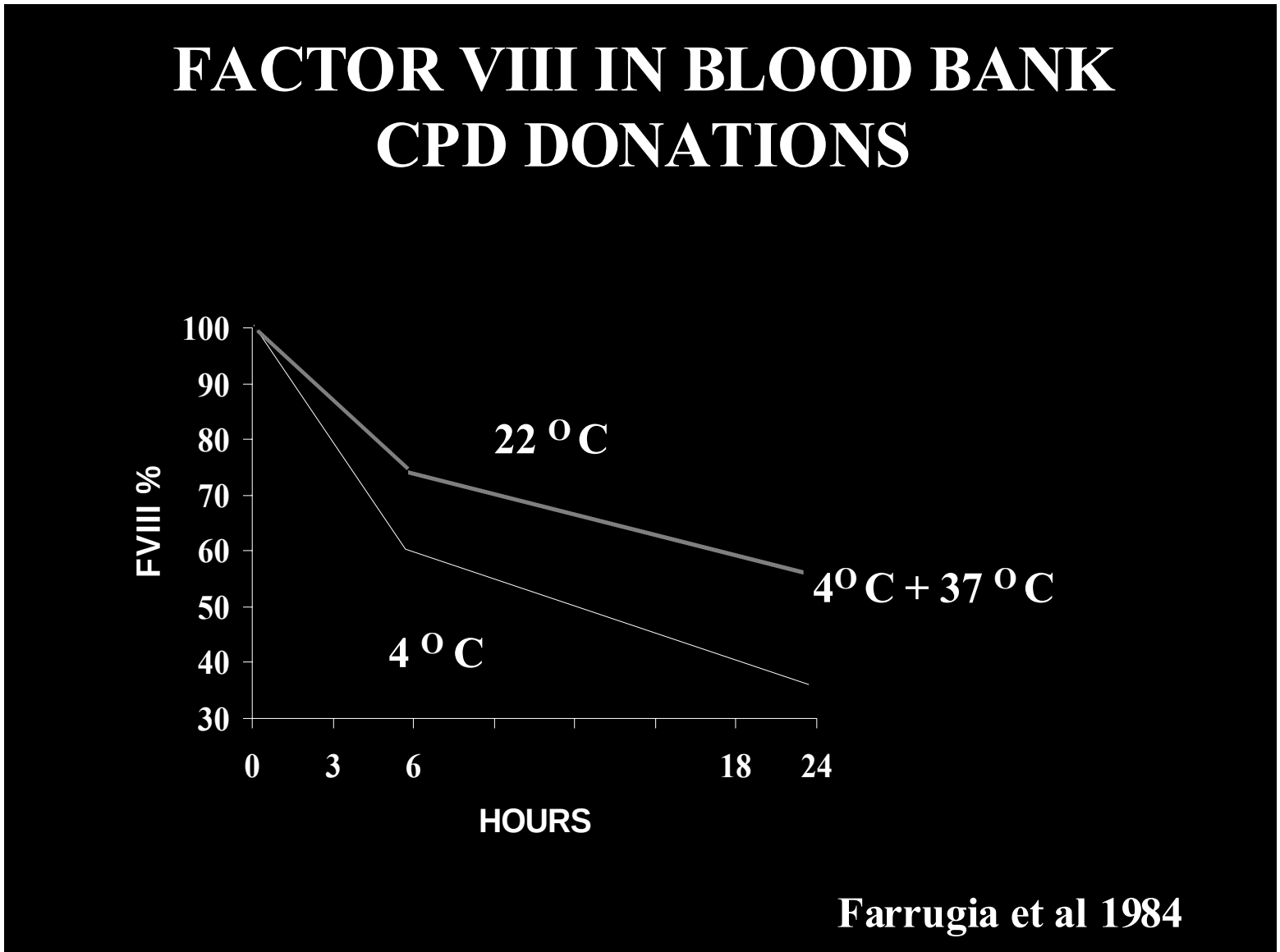
(for fractionation)

On a pool of not fewer than ten units, measurement of factor VIII, using the EP reference method and a reference plasma calibrated against the International Standard for blood coagulation factor VIII in plasma. The activity is not less than 0.7 I.U. per millilitre.

Factors claimed to affect FVIII yield in fractionated concentrates

- Anticoagulant
- Collection method
- Time/Temperature to separation/freezing
- Freezing rate
- Storage conditions of frozen plasma
- Thawing conditions
- Purification chemistry
- Viral inactivation

Plasma quality
The role of Factor VIII



Influence of blood cooling on FVIII in plasma

Cooling temperature	FVIII IU/ml	Protein g/l
0-4°C n=9	0.45±0.06	63.7±2.2
20°C n=9	0.84±0.1	65.6±2.7

Over et al (1990) Dev Hem & Imm 24:11-24

Factors claimed to affect FVIII yield in fractionated concentrates

- Anticoagulant
- ***Collection method***
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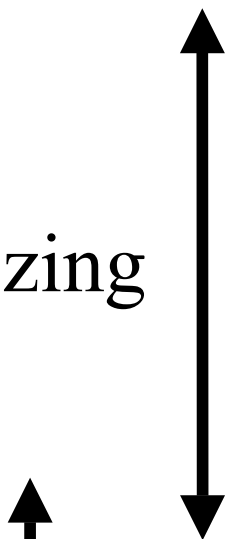


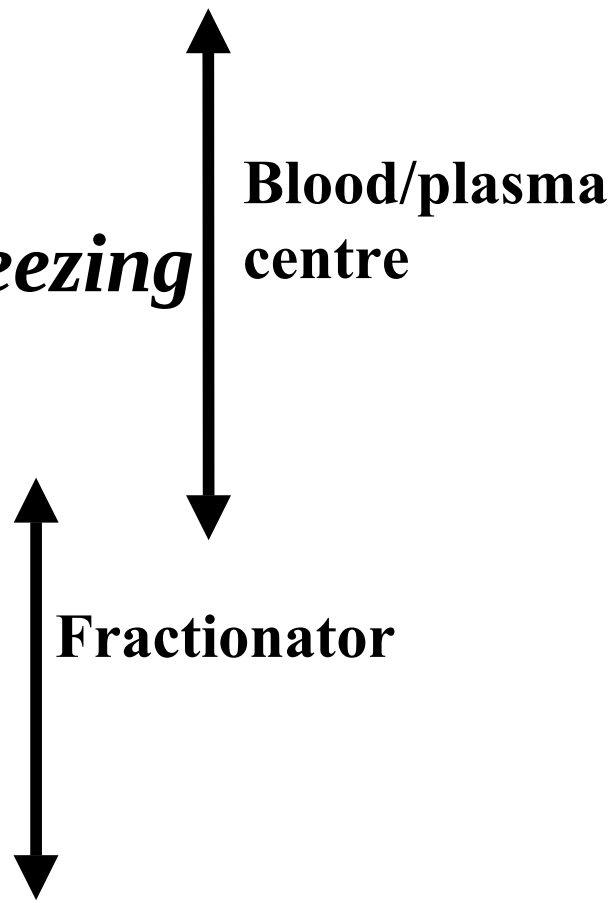
Source vs recovered plasma
FVIII yield in low purity concentrates

	Cryo g/kg plasma	**FVIII IU/kg plasma**	
		Cryo extract	**Pre-finish**
Manual <18 h, CPDA n=13[1]	9.1±0.5	391±31	290±25
Haemonetics CPD[1]	10.9±1.1	461±50	319±33
Whole blood[2]		403	283
Apheresis[2]		450	317
Apheresis/low citrate[2]		507	362

[1]Smith et al (1985) - Low purity concentrate
[2]Ribeiro et al 1997 - Intermediate purity concentrate

Factors claimed to affect FVIII yield in fractionated concentrates

- Anticoagulant
- Collection method
- ***Time/Temperature to separation/freezing***
- Freezing rate
- Storage conditions of frozen plasma
- Thawing conditions
- Purification chemistry
- Viral inactivation



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DONATION-FREEZING INTERVAL
EFFECT ON FVIII LEVELS
ARCBS data mid 1990's

DELAY TO FREEZING	% < 0.7 IU/mL
APHERESIS < 12 HOURS	1%
WHOLE BLOOD < 12 HOURS	13%
WHOLE BLOOD < 18 HOURS	27%
WHOLE BLOOD < 24 HOURS	40%

Factor VIII content of 5 litre plasma packs of different ages

	Factor VIII (IU/kg) in plasma samples		
Time (h) between blood collection and plasma freezing	**Before freezing pack**	**After thawing pack to 20°C**	**Core sample of frozen pack**
3-4 - special collection	930	870	880
4-8 - routine fresh frozen	840	820	790
16-18 - routine overnight frozen	800	740	730

Smith et al (1985) Dev Hem & Imm 13 :15-23

Effect of pack type and freezing rate on plasma FVIII

	Plasma IU/ml	
	6-8 hours	**18 hours**
5 L packs over ≈ 2 h	0.84±0.13 (n=10)	0.77±0.08 (n=10)
SD packs over ≈ 15 min	0.86±0.10 (n=10)	0.77±0.15 (n=10)

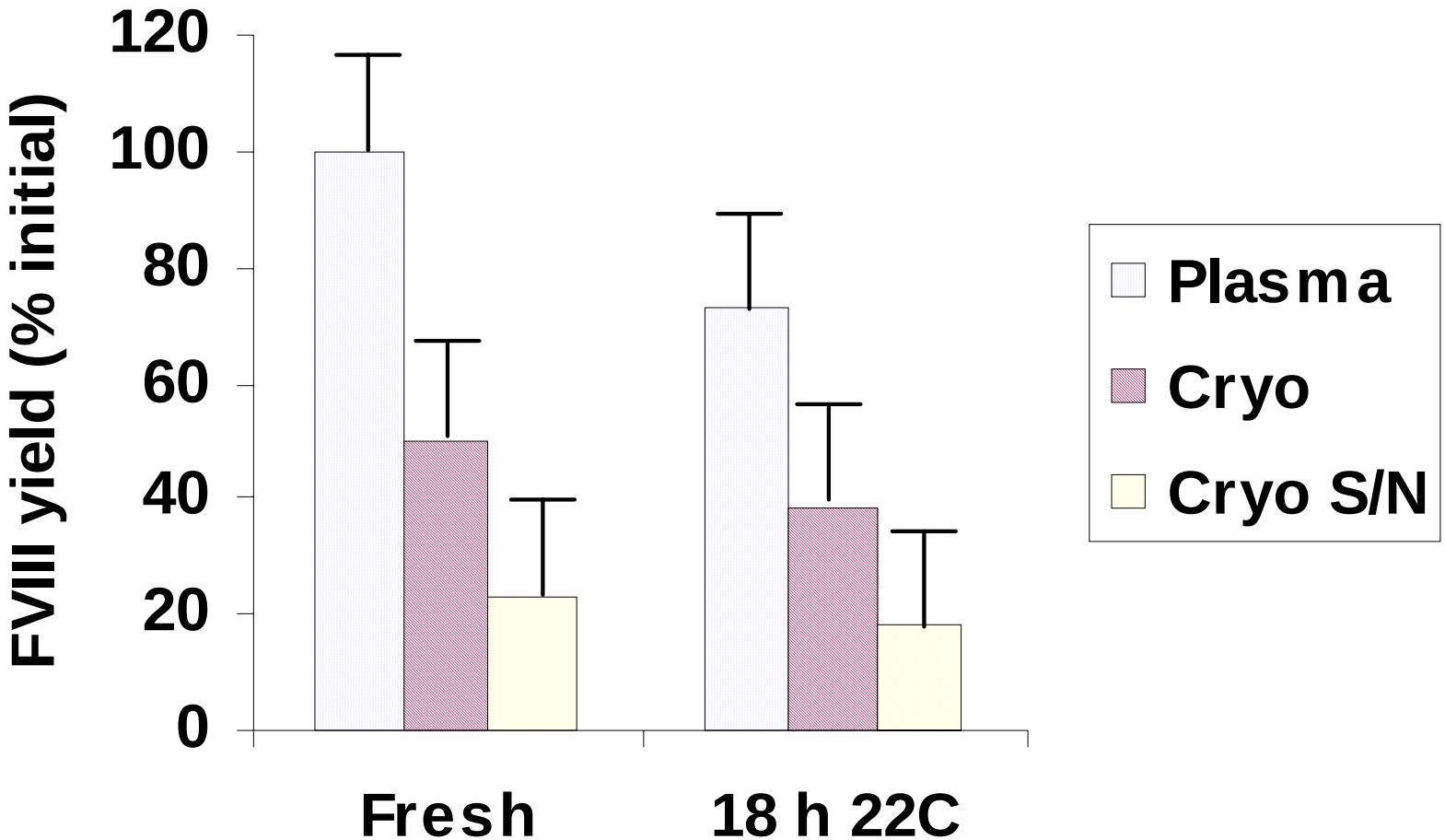
Smith et al (1985) Dev Hem & Imm 13 :15-23

DOES IT MATTER?

- There is no doubt that delayed blood processing to frozen plasma decreases FVIII levels in plasma for fractionation
- Does this affect the yields and quality of fractionated products?

Distribution of FVIII in small scale plasma cryoprecipitation

Effect of overnight storage



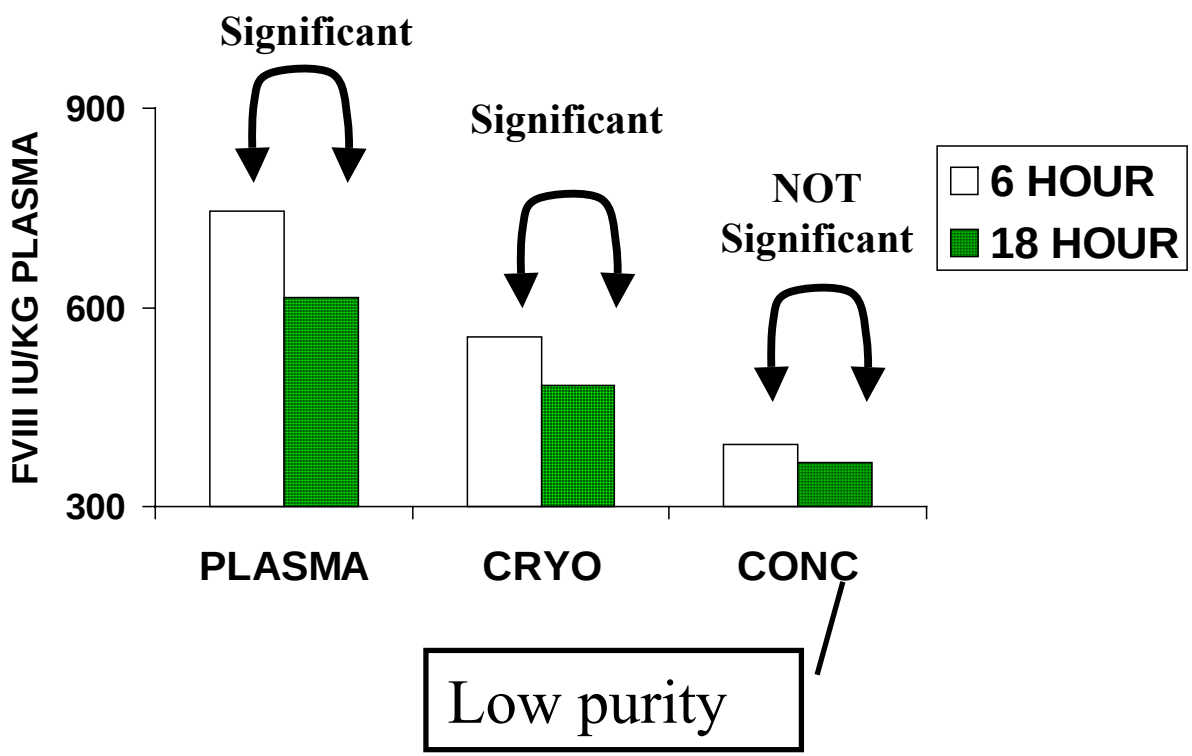
Hellings et al (1982)

Plasma Quality

Effect of delayed freezing on FVII

Hughes et al 1989

"……. *plasma intended for the recovery of proteins that are labile in plasma is frozen by cooling rapidly at – 30 °C or below as soon as possible and at the latest within 24 h of collection.*" EP Monograph



Effect of separation/freezing interval on FVIII yield in low purity concentrates

	FVIII recovery IU/kg plasma			
	ACD		CPD	
	6-8 hours	**18 hours**	**6-8 hours**	**18 hours**
Cores of frozen packs (mean of 10 cores of five L packs)[1]	720	700	840	770
Freeze-dried concentrate (mean of 8 batches) [1]	214	208	255	257
Continuous thaw[2]			293	268
Batch thaw[2]			204	174

[1] Smith et al (1985) Dev Hem & Imm 13 :15-23
[1] Foster et al (1985) Dev Hem & Imm 13 :15-23

DOES IT MATTER?

- There is no doubt that delayed blood processing to frozen plasma decreases FVIII levels in plasma for fractionation
- Does this affect the yields and quality of fractionated products?

IT DEPENDS

- Cryo yield affected
- LP & IP sometimes affected
- No data for current generation of FVIII concs

Factors claimed to affect FVIII yield in fractionated concentrates

- Anticoagulant
- Collection method
- Time/Temperature to separation/freezing
- ***Freezing rate***
- Storage conditions of frozen plasma
- Thawing conditions
- Purification chemistry
- Viral inactivation

"Plasma should be frozen at…….

…….-18^{O}C, -20^{O}C, -23^{O}C,-30^{O}C?"

- Remarkably ambiguous language in standards
  - "*cooling rapidly at -30^{O}C, frozen at -20^{O}C"* (EP)
  - "*shall be stored at a temperature not warmer than -20^{O}C"* (CFR)
- Little recognition of the important - obvious - parameter

THE FREEZING ***RATE***

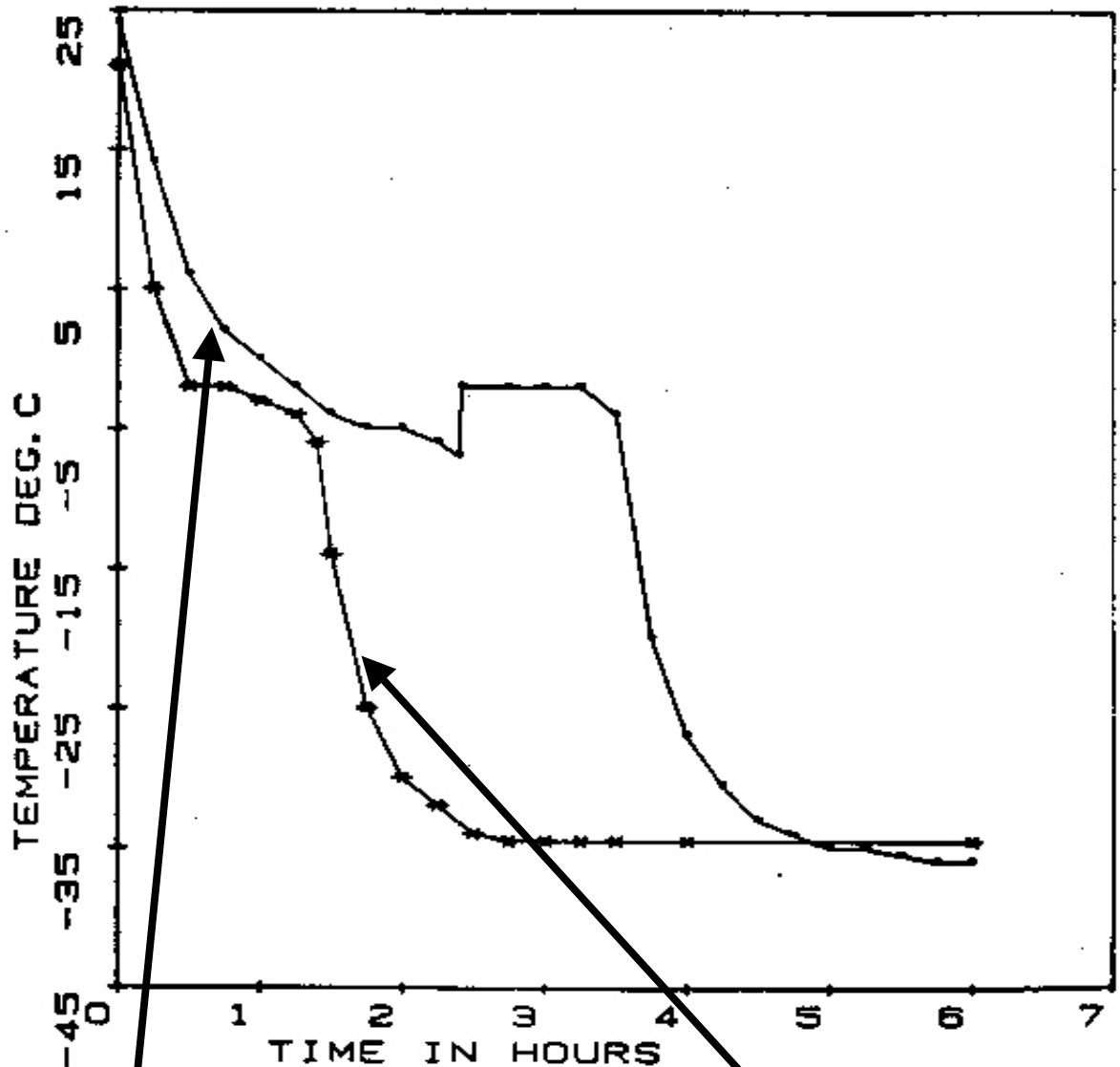
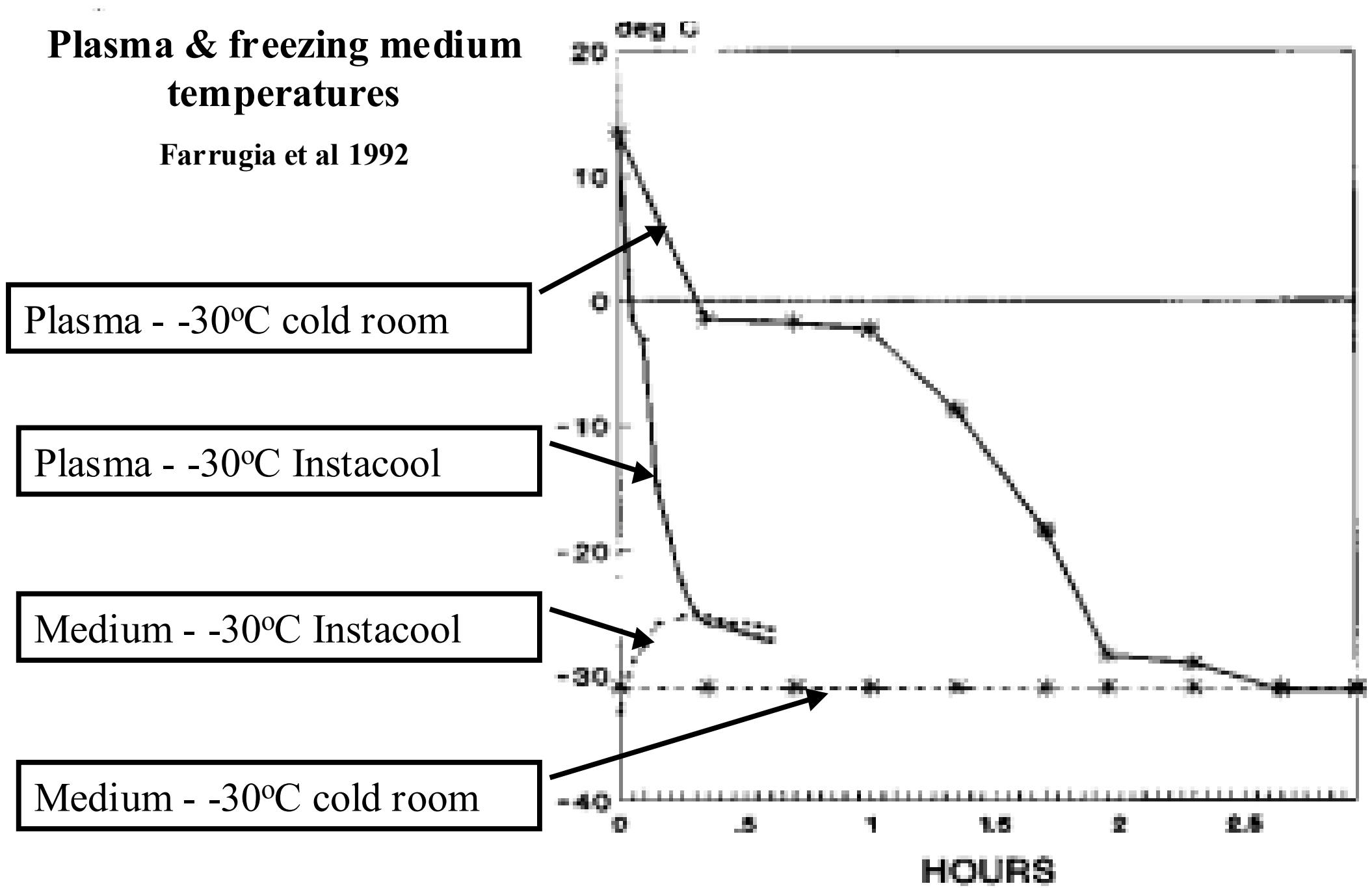


Figure 4. Freezing and supercooling in plasma. **: Plasma placed directly on −50°C cold shelf; ●●: Plasma cooled to below 0°C on a −13°C shelf, then shelf temperature reduced to −50°C.

McIntosh et al (1990) Dev Hem & Imm 24:11-24)

Plasma & freezing medium temperatures

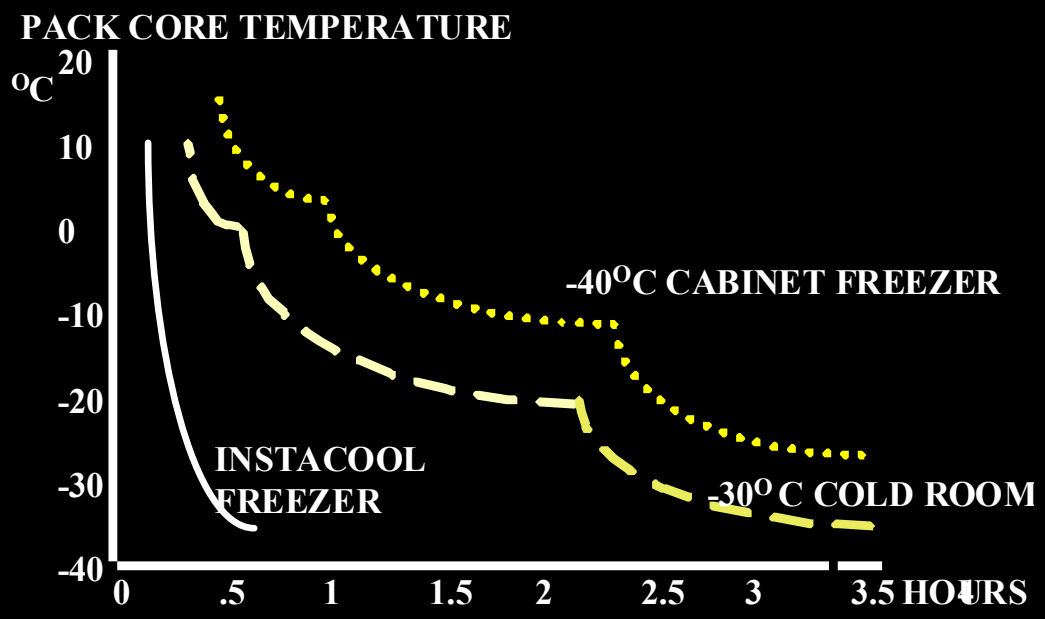
Farrugia et al 1992



Plasma freezing rates and FVIII

PLASMA FREEZING RATES

Farrugia et al 1992



FVIII YIELDS

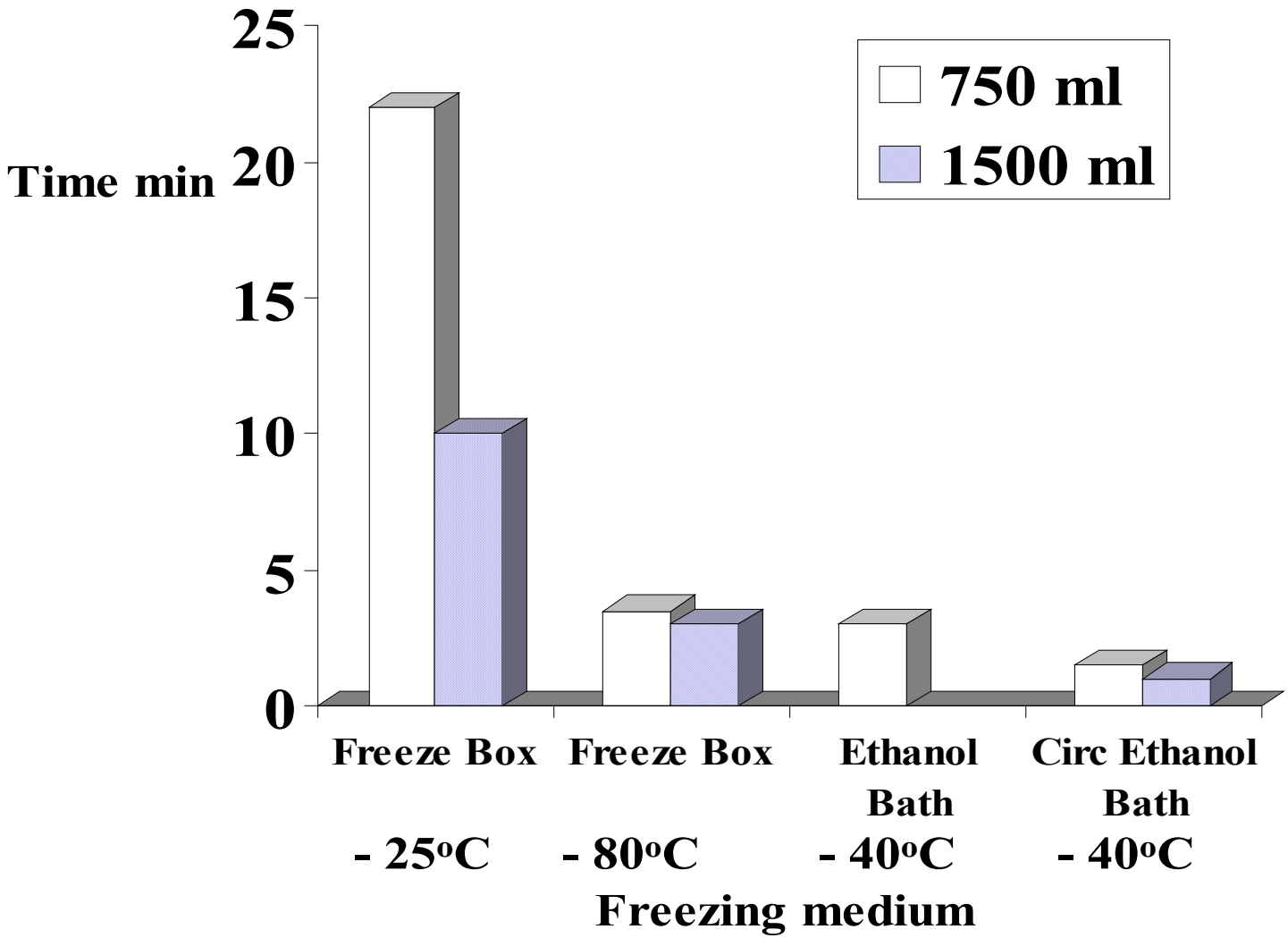
EFFECT OF PLASMA FREEZING RATE

FREEZING MEDIUM	FVIII U/KG (Blood Bank CRYO)
(1) INSTACOOL FREEZER	575±122
(2) - 30 °C COLD ROOM	443±62
(3) -40 °C CABINET FREEZER	303±130 *

* P<0.01 vs (1) & (2)

Farrugia et al 1992

Plasma freezing time to -25°C with different equipment



Carlebjőrk et al (1986) Transfusion 26:159-162

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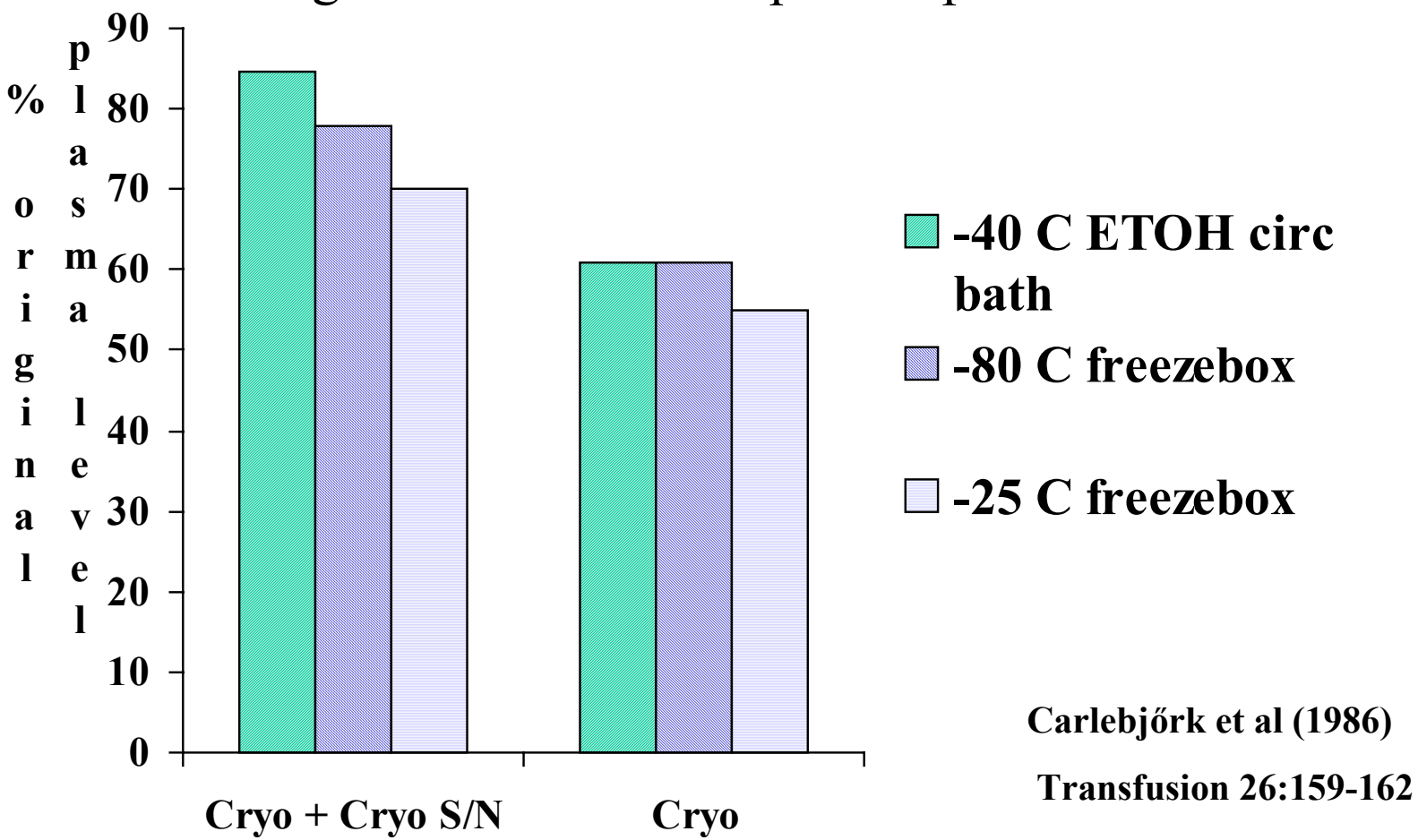
FVIII recovery in cryoprecipitate and cryosupernatant with different freezing methods

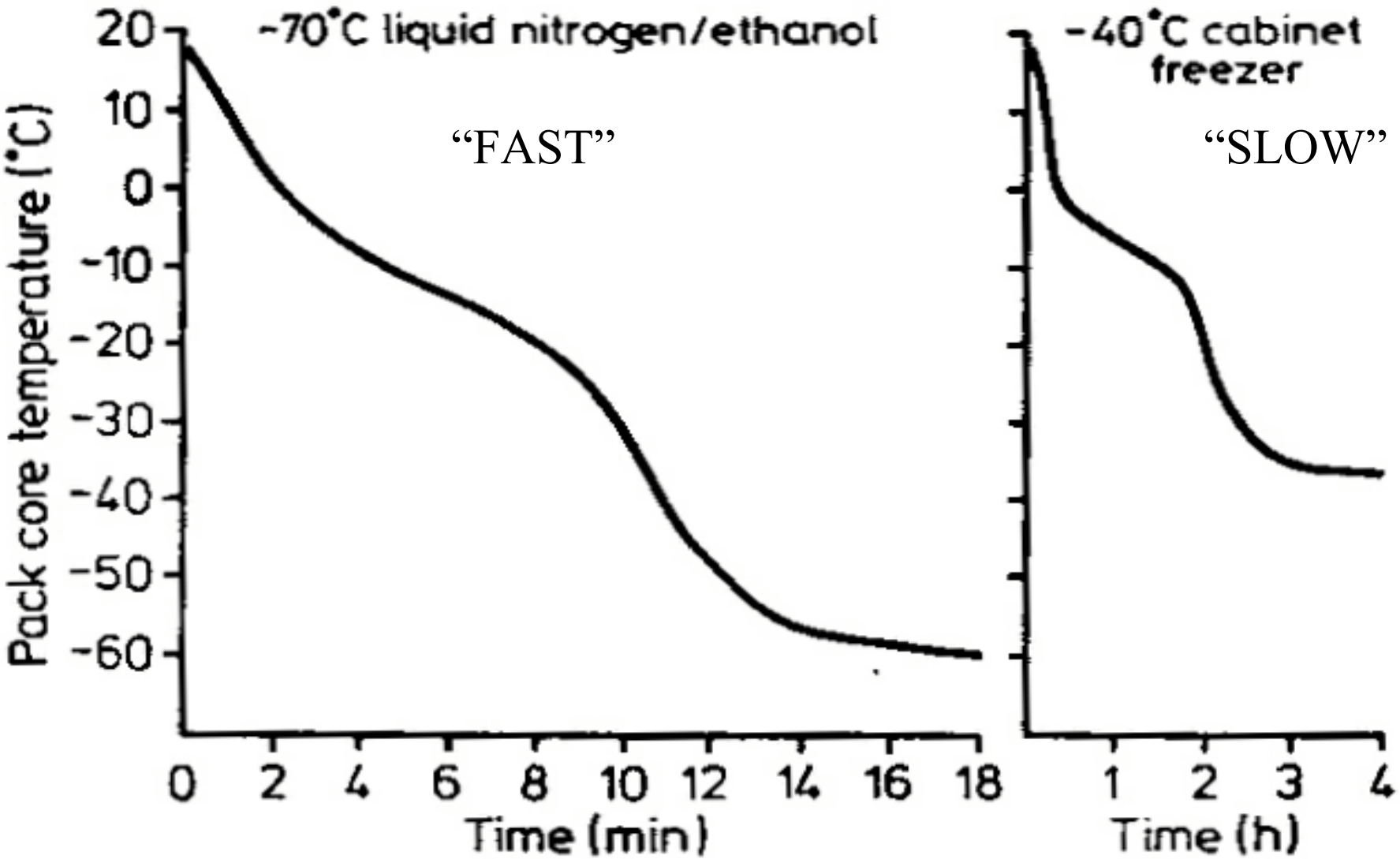
Average results from four plasma pools



Carlebjőrk et al (1986)

Transfusion 26:159-162

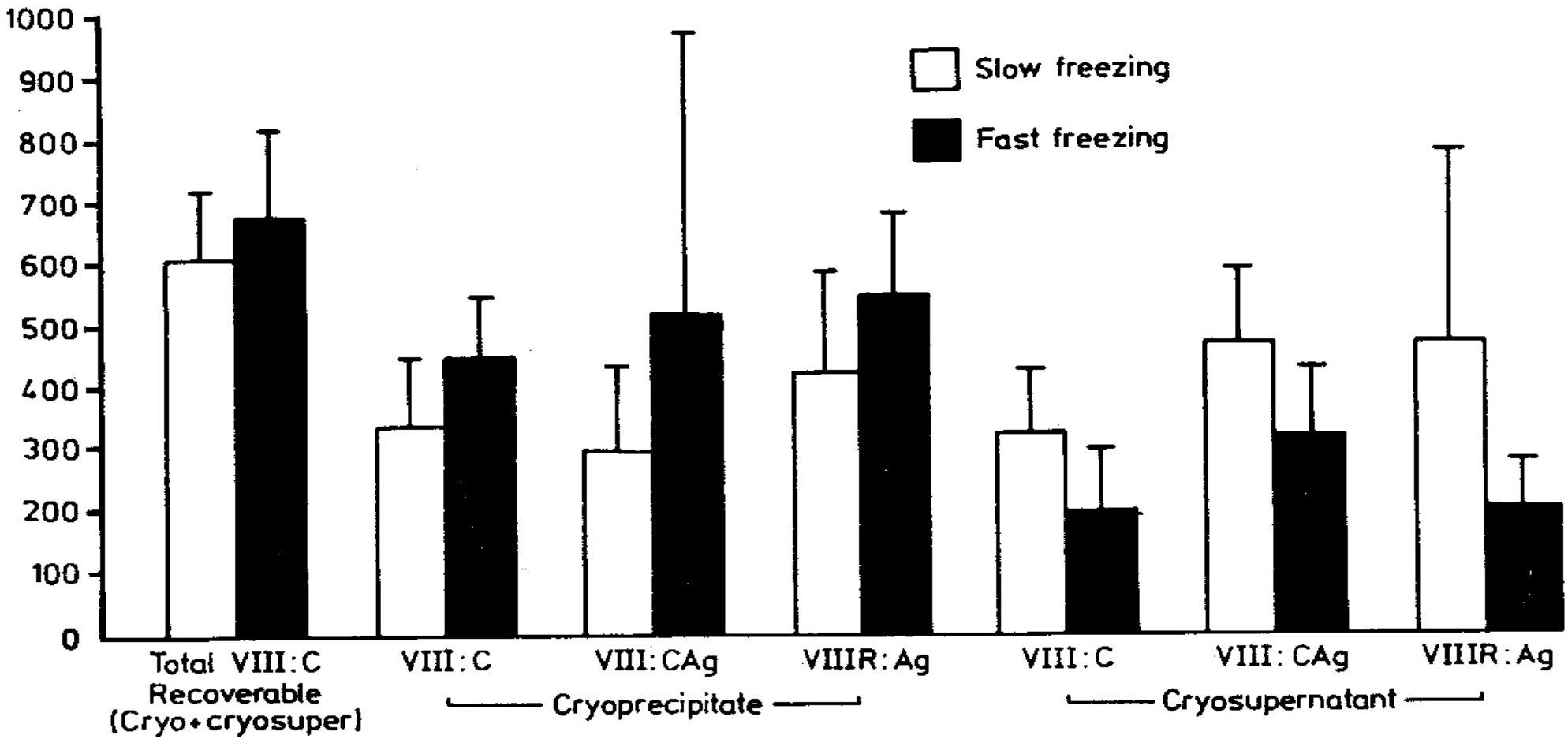
Plasma frozen in different media



Farrugia and Prowse (1985) J Clin Path 38: 433-437

FVIII related activities in plasma fractions

Thaw-siphon cryoprecipitation - effect of plasma freezing rate



Farrugia and Prowse (1985) J Clin Path 38: 433-437

Effect of freezing rate on cryoprecipitate quality

Freezing condition medium	Temp	Thickness of plasma layers		FVIII IU/L	Fibrinogen g/L	Total protein g/L
		4 cm	2 cm			
Alcohol dry ice	-70ºC	11 min	7 min	467	10.5	23.2
Circulating N_2-gas	-100 ºC	12 min	10 min - fast	513	11.2	23.7
Stationary air	-30 ºC	4 hours	3 hours	490	13.0	29.5
Idem + insulation	-30 ºC	19 hours	15 hours - slow	433	14.6	34.9

Over et al (1985) Dev Hem & Imm 13 :67-78

Plasma freezing
What is important?

- Rapid freezing - ca -30^{o}C in 30 minutes - results in better FVIII yields in cryo relative to slower freezing - - ca -30^{o}C in 3-4 hours
- The ice crystal structure and the physical nature of cryoprecipitate are affected by the plasma freezing rate.
- Slower freezing also increases fibrinogen in cryo; this has its pro's and con's
- The effect of freezing rates on FVIII yields in current concentrates is not well recorded

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Factors claimed to affect FVIII yield in fractionated concentrates

- Anticoagulant
- Collection method
- Time/Temperature to separation/freezing
- Freezing rate
- ***Storage conditions of frozen plasma***
- Thawing conditions
- Purification chemistry
- Viral inactivation

Blood/plasma centre

Fractionator

Plasma freezing and storage Effect on thaw siphon blood bank cryoprecipitate

Freezing	Storage period	Storage temperature	FVIII cryo yield IU/kg plasma	Fibrinogen in cryo mg/kg plasma
Fast	16 h	- 20 ° C	426	605
	3 mo		500	609
	6 m		416	538
	16 h	- 40 ° C	493	607
	3 mo		522	577
	6 mo		449	542
Slow	16 h		318	522
	3 mo		306	502

Farrugia and Prowse (1985) J Clin Path 38: 433-437

Effect of variation in plasma cold storage on FVIII recovery and cryoprecipitate quality

	Cold Storage ºC	
	-40 ºC to -20 ºC (1 month) to -40 ºC	**-40 ºC**
Number of batches	5	9
Batch size (L)	751±80	718±77
Plasma FVIII (IU/L)	795±84	790±77
Cryoprecipitate weight (g/L plasma)	11.63±0.48	10.98±0.57
Cryoprecipitate extract FVIII (IU/L plasma)	597±25	594±40
Cryoprecipitate quality (IU/L)	51.4±2.7	54.2±2.3

McIntosh et al (1990) Dev Hem & Imm 24:11-24)

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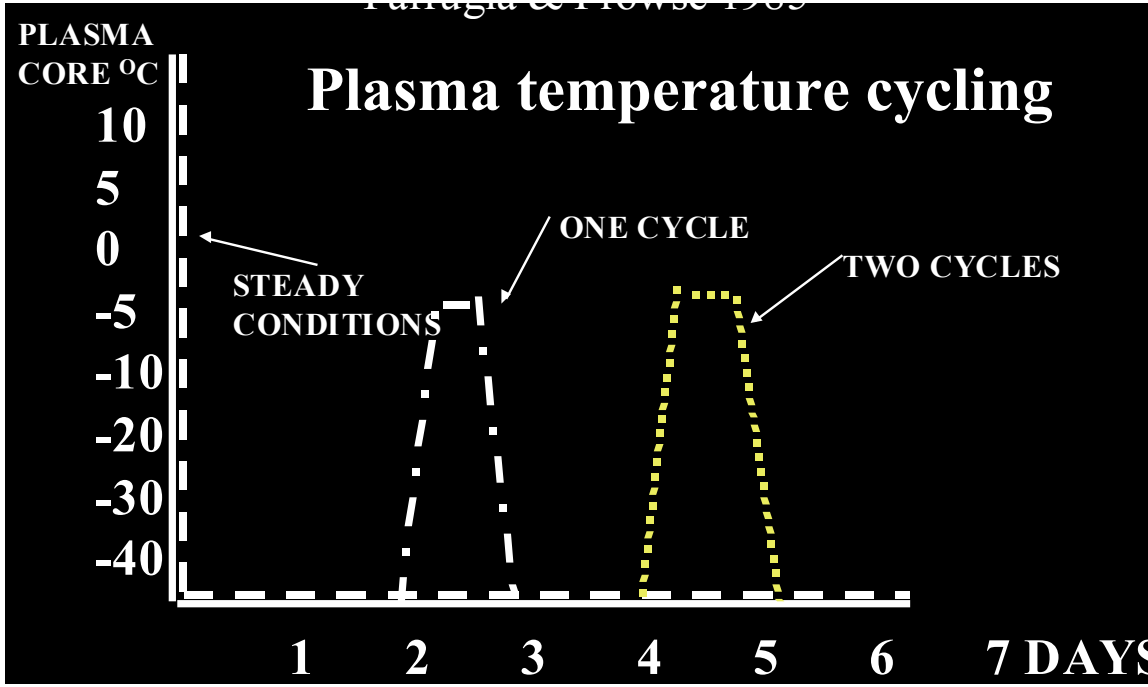
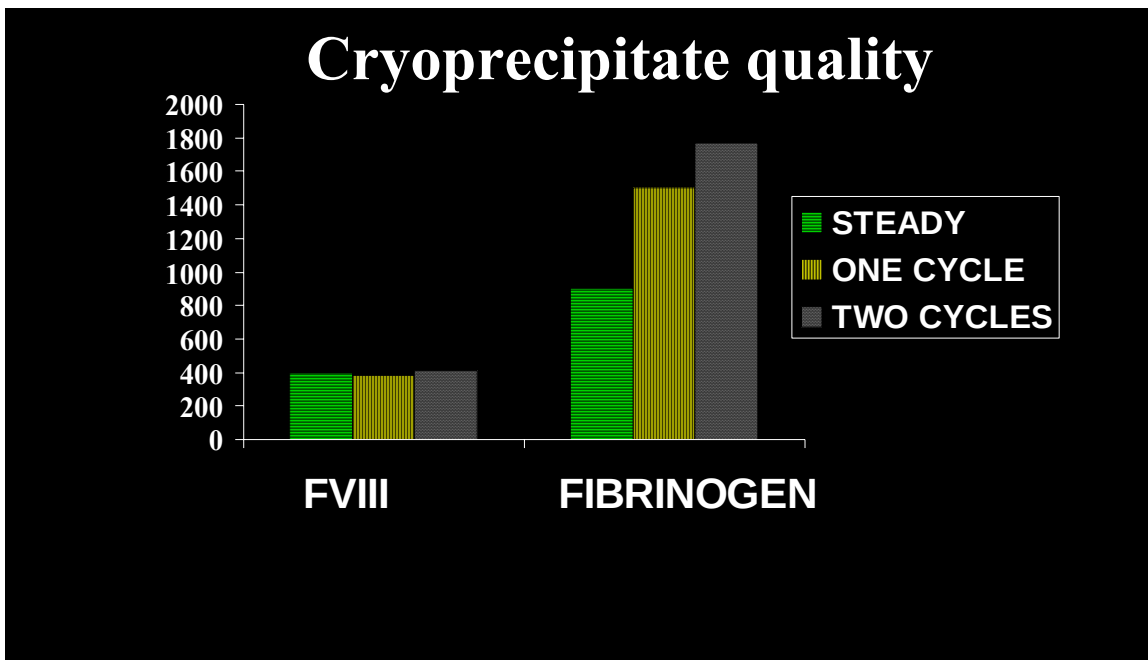
Plasma Quality

Effect of poor storage conditions

"…….*Source Plasma intended for manufacture into injectable products that is inadvertently exposed (i.e., an unforeseen occurrence in spite of compliance with good manufacturing practice) to a storage temperature warmer than -20 deg.C and colder than +10 deg.C may be issued only if labeled as ``Source Plasma Salvaged.''"* CFR 21- 640





Effect of thawing and re-freezing CPD plasma on plasma FVIII and concentrate yield

	Stage yield IU/kg		
Stage	**Once-frozen**	**Twice-frozen**	**Significance**
Plasma cores (plasma standard)	593±99	301±116	Significant
Cryo extract (conc. Standard)	330±35	291±35	Not significant
Dried low purity concentrate	194±19	192±19	Not significant

Smith et al (1985) Dev Hem & Imm 13 :15-23

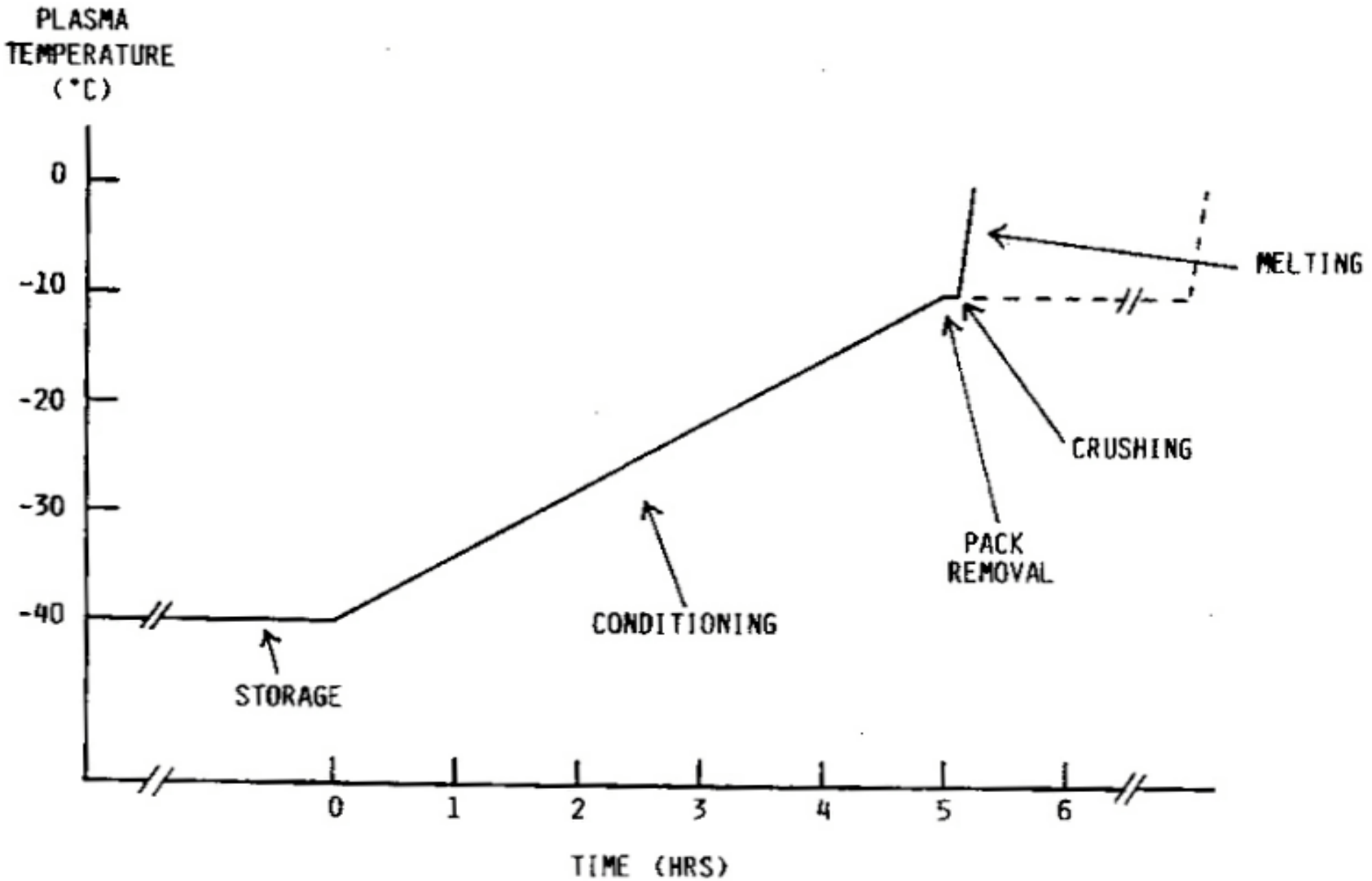
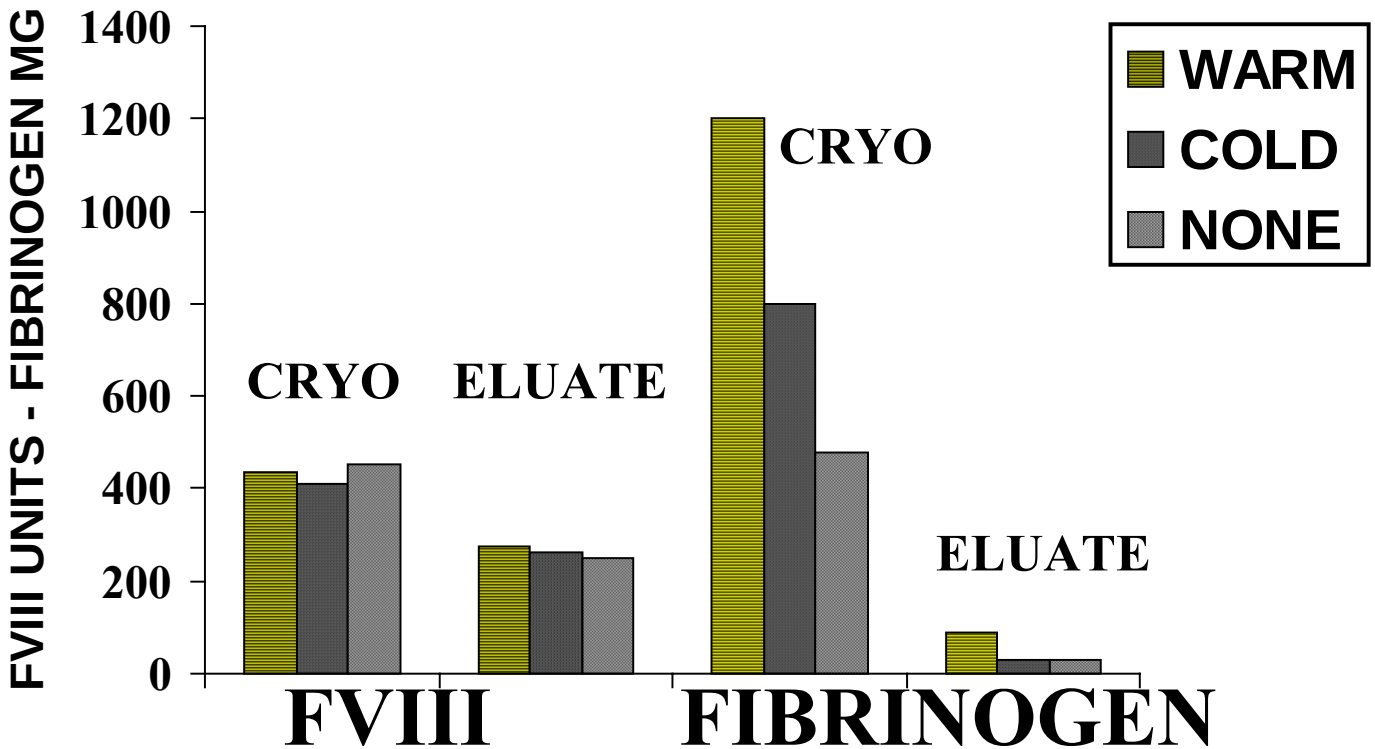


Figure 1. Schematic representation of the present Scottish procedure for thawing plasma.

Foster et al (1985) Dev Hem & Imm 13 :15-23

Plasma conditioning

Effect on FVIII concentrate



Farrugia et al (1992) Transfusion 32:755-759

Effect of plasma conditioning on cryoprecipitate FVIII

Conditioning regimens				
▪ PCU temperature	-10 ºC	-15 ºC	+4 ºC	-16 ºC
▪ Conditioning time	6 hours	6 hours	2 hours	5 hours
▪ Plasma temp at crushing	-8 ºC	-12 ºC	-10±2 ºC	-10.5±0.5 ºC
▪ Plasma storage temp	-20 ºC	-20 ºC	-40 ºC	-40 ºC
Number of batches	18	5	36	13
Batch size L	750±54	789±84	761±104	785±129
Plasma FVIII (IU/L)	770±68	789±84	764±130	769±72
Cryoprecipitate weight g/L plasma)	12.21±1.19	11.63±0.48	10.7±0.64	10.05±0.34
Cryoprecipitate extract FVIII (IU/L plasma)	567±47	597±24.8	509±42	551±50.3
Cryoprecipitate quality (IU/g)	46.4±4.1	51.4±2.7	47.8±4.6	54.4±5.5

McIntosh et al (1990) Dev Hem & Imm 24:11-24)

Plasma conditioning

Effect on blood bank cryoprecipitate

Plasma processing conditions	FVIII IU	Fibrinogen mg	Fibronectin mg	Adhesive strength g	VWF U
Stored -30ºC, immediately thawed in 4 ºC WB	132±24	213±82	53±21	7.6±1.8	189±31
Stored -30 ºC, then 4 ºC cold room (in polystyrene box) for 18 h, then thawed in 4 ºC WB	110±22	457±125 $p<0.001$	63±17	23.5±4.8 $p<0.05$	221±25

Farrugia et al (1992) Transfusion 32:755-759

Plasma Storage
What is important?

- As long as freezing is optimised, storage requirements appear to be flexible in the range -20^{o}C to -40^{o}C
- Maintaining a steady storage temperature is more important than the absolute storage temperature, within this range
- While temperature changes can affect the quality of cryoprecipitate, this can be exploited to improve both blood bank and industrial cryo

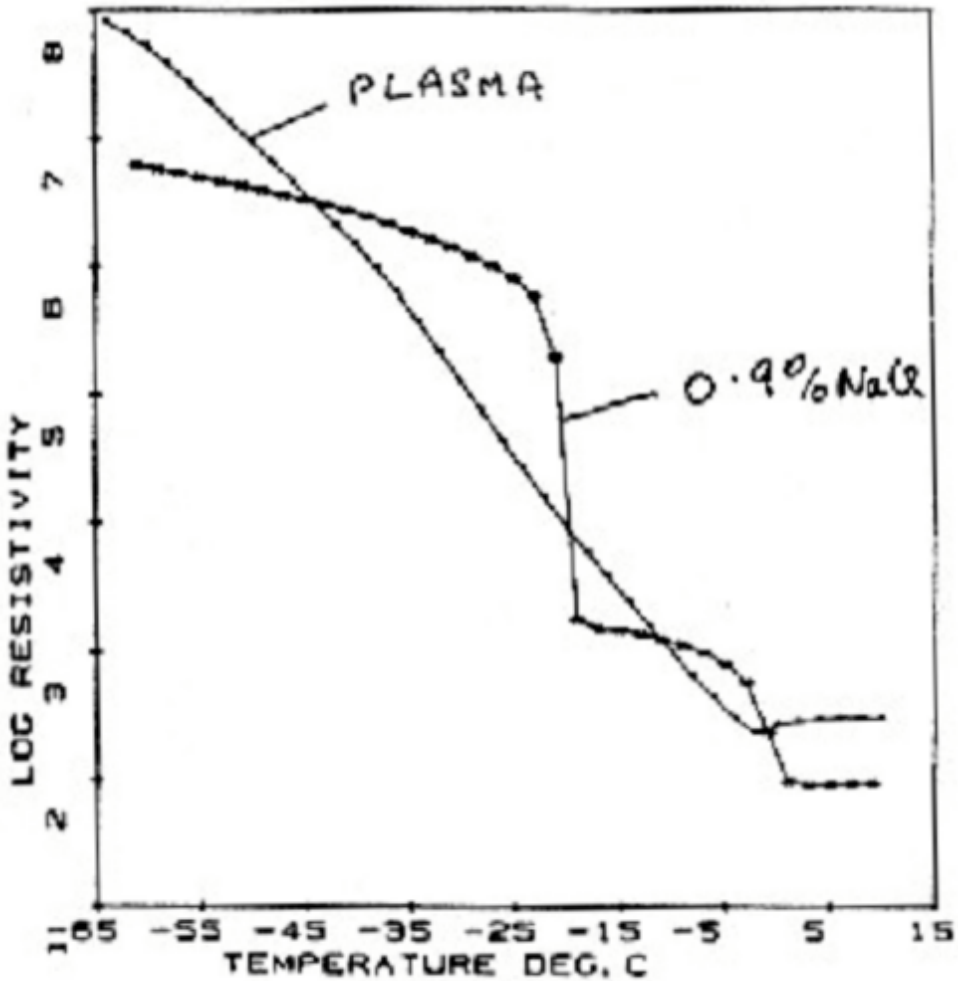
The “theory” of plasma freezing - *CBBS e-Network Forum*

A transfusion medicine physician in the Netherlands *reports that the optimal storage temperature of fresh frozen plasma is* ***minus 30 degrees*** *centigrade or colder as is generally agreed upon in Europe and as can be read in " The guide to the preparation, use and quality assurance of blood components", Council of Europe Publishing, ISBN 92-871-3530-4. The scientific background of this choice is the following:*

“Plasma is a solution of proteins and salt in water. Freezing of such a solution results in the formation of pure water ice until the ***eutectic*** *freezing point at minus 23 degrees centrigrade is reached and then also the solutes like proteins and salts start to form crystals. Finally, the total plasma mixture has frozen solid after the eutectic point temperature has been reached. During this freezing process the remaining liquid gradually becomes an ever more concentrated solution of the salts and proteins…..Labile proteins like factor VIII and other clotting enzymes will denature when exposed for a long time to these highly concentrated acid salt solutions. During the frozen storage, the freezer with plasma will be opened and closed regularly in a blood bank, and increases in temperature inside a freezer of up to 5 degrees centigrade can easily occur. At any temperature higher than minus 23 degrees centrigrade the original eutectic mixture of salts, proteins and water will become liquid again and the deterioration of the labile proteins will resume. If you keep the average storage temperature of the frozen plasma below minus 30 degrees centigrade, you avoid the critical eutectic temperature and the plasma will maintain its original quality.”*

Oh yeah?

Eutectic point of plasma?



Resistivity of normal plasma & saline measurements on slow thawing after fast freezing
McIntosh 1990

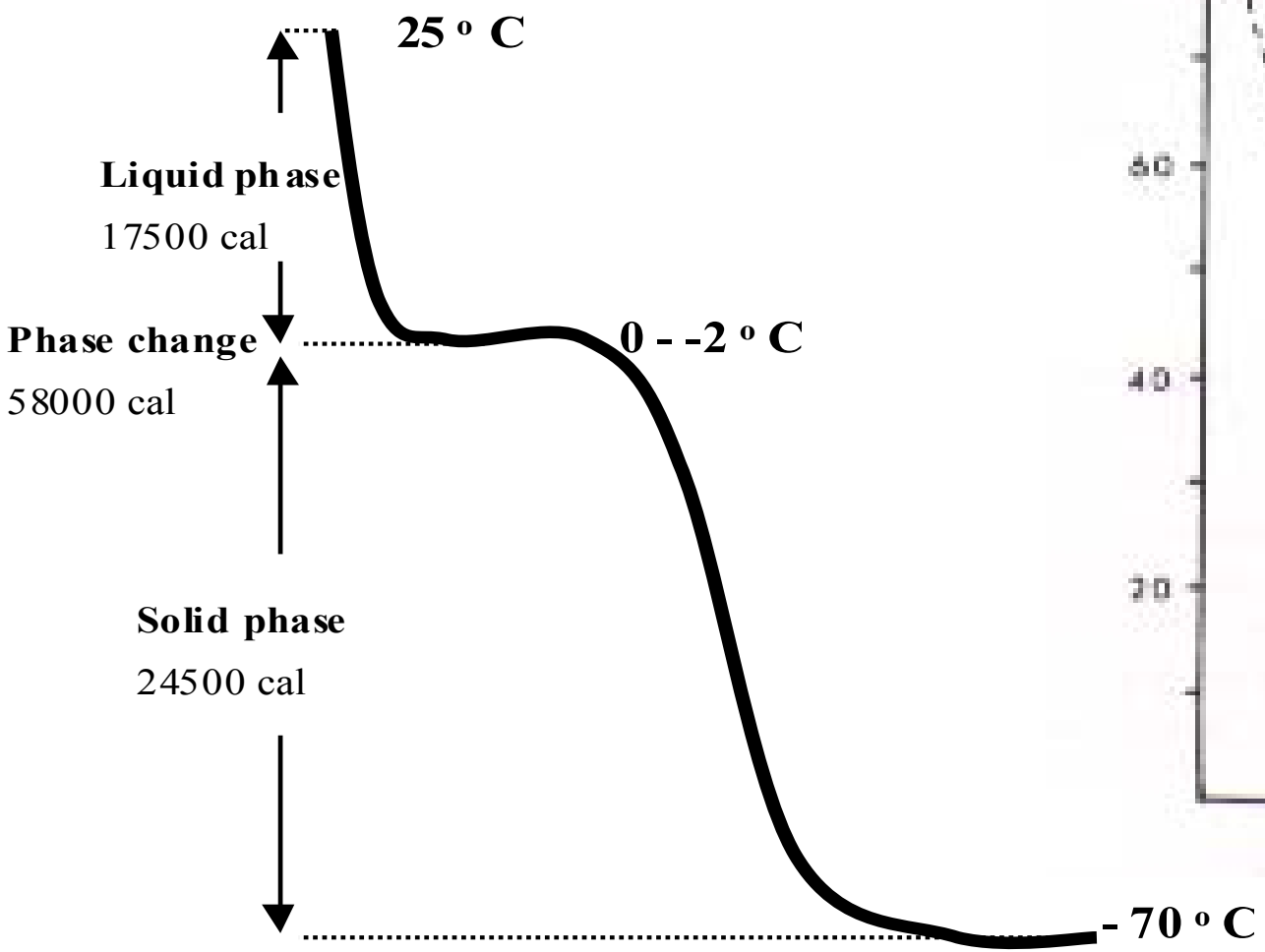
Transition	Temperature of transition (^{o}C)	
	Slow freezing (2^{o}C/min)	Rapid freezing (200^{o}C/min)
1 Glass transition (onset of motion of water molecules)	- 80	- 85
2 Antemelting (onset of molecular mobility of proteins)	- 42 to –38	- 38 to –35
3 Incipient melting (beginning of thermodynamic melting of ice)	- 27	- 27
4 Melting point (final melting of ice)	- 0.5	- 0.5
MacKenzieAP 1980		

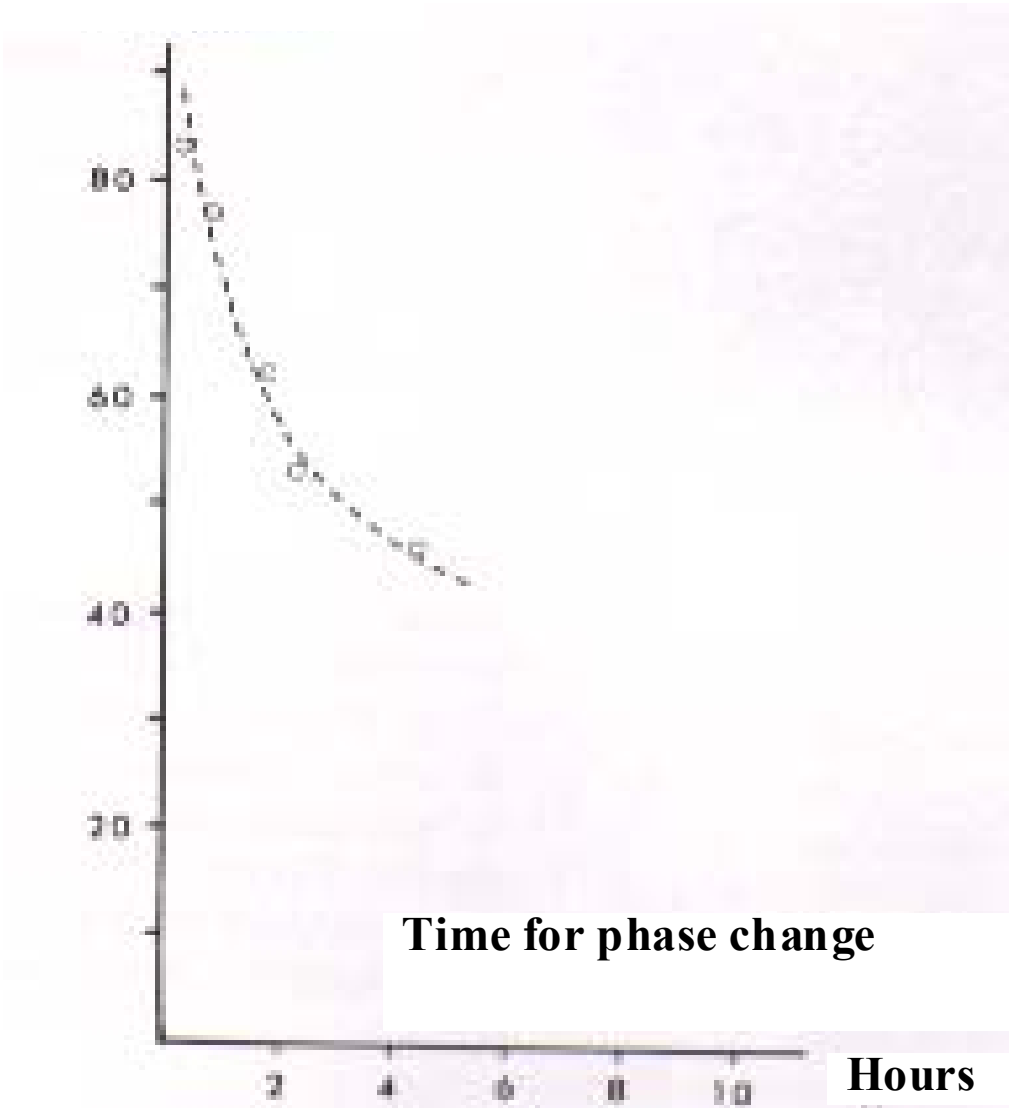
Phase transitions in frozen plasma

Freezing of 700 ml plasma

Energy consumption at different stages of freezing

Carlebjőrk et al (1986) Transfusion 26:159-162





Plasma freezing and storage

- Conventional eutectics offer no guidance
- Freezing so that phase change is as rapid as possible
- Storage so that this is maintained - -20^{o}C is adequate

⇒AND WHY SHOULD THIS BE AN ISSUE FOR REGLATORS ANYWAY?

⇒IS THERE ANY EVIDENCE THAT BLOOD/PLASMA PROCESSING AFFECTS SAFETY AND QUALITY (AS OPPOSED TO YIELD)?

FVIII and activation of coagulation in plasma freezing

Split 300 ml pairs from 600 ml source plasma units, n=12

	-30°C	-80°C	% difference	p
FVIII:C (u/dL)	103 (84-16)	121 (108-149)	16	0.0005
FVIII:Chr (U/dL)	96 (85-107)	105 (90-117)	8.9	0.0005
C:Chr ratio	1.05 (0.88-1.21)	1.15 (1.05-1.43)	9.0	0.0005
F1+2 (nmol/L)	0.60 (0.36-1.74)	0.84 (0.43-2.11)	33.3	0.001

NB - ***FAST*** FREEZING RESULTS IN ***HIGHER*** ACTIVATION

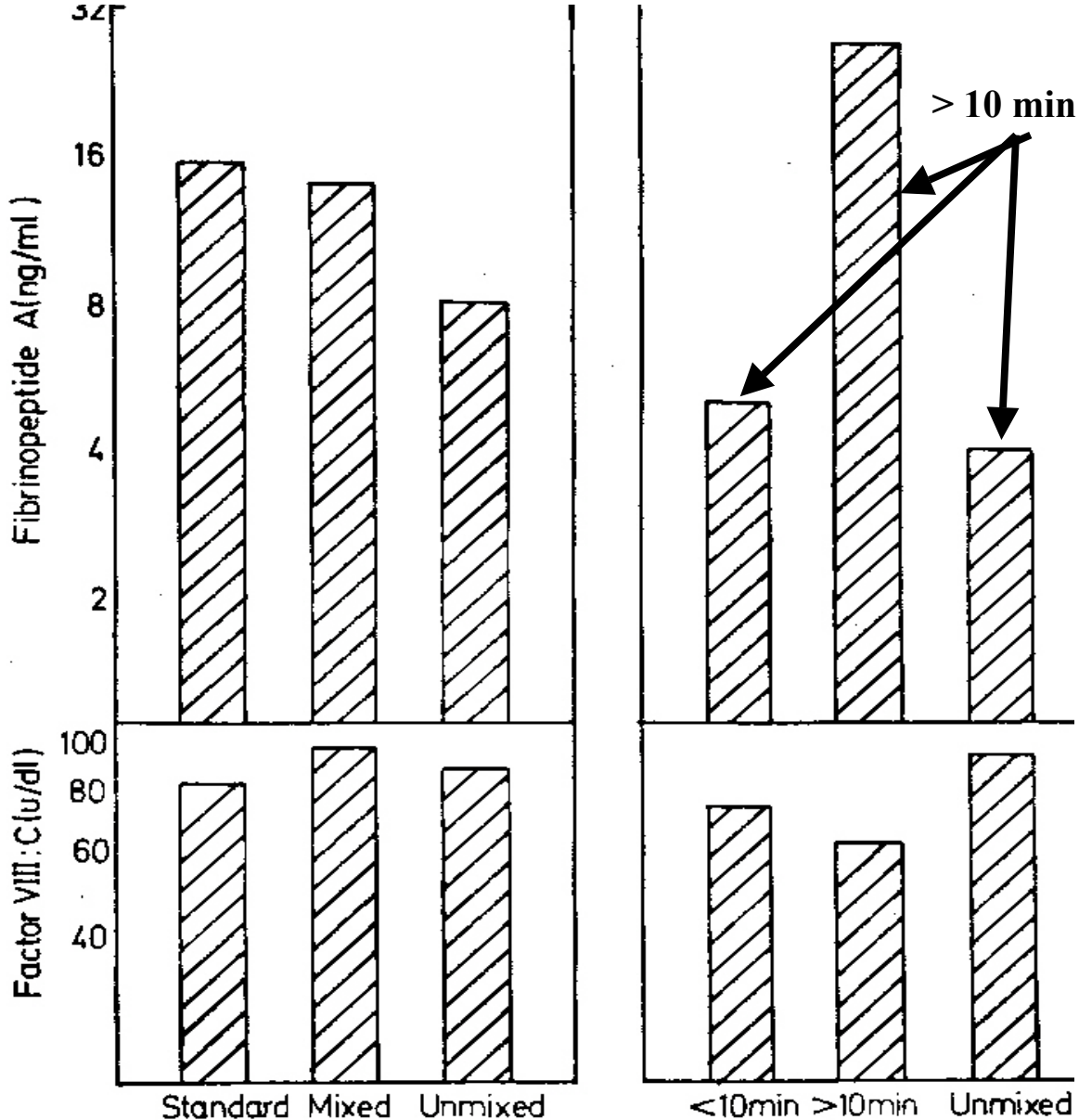
Hellstern et al (2001) Trans 41:1601-1605

FVIII from concentrates made from plasma pools with evidence of coagulation activation

	Normal coagulation markers	**Elevated coagulation markers**
FPA in plasma pools	1.7≤ FPA≤4.5 μg/ml	11.2≤ FPA≤13.4 μg/ml
TAT in plasma pools	2.1≤ TAT≤3.0 ng/ml	11.4≤ TAT≤15.9 ng/ml
40 kDa fragment FVIII heavy chain fragment	Absent	Present
Inhibitor development	None of the batches resulted in inhibitors	All inhibitor patients received these batches
Binding of FVIII in product to PS/PC	156	30
Binding of FVIII in product to mcab to C2 domain overlapping with VWF/PL	156	30
Binding of FVIII in product to mcab to C2 domain not overlapping with VWF/PL	13	15

Saeneko et al 2001Thrombosis Research 101:501-511

Effect of mixing and donation time on plasma FpA



Blood processing and activation of coagulation

- Standard blood collection measures minimise activation
- No special measures are needed
- Reported FpA levels leading to problems are abnormally high

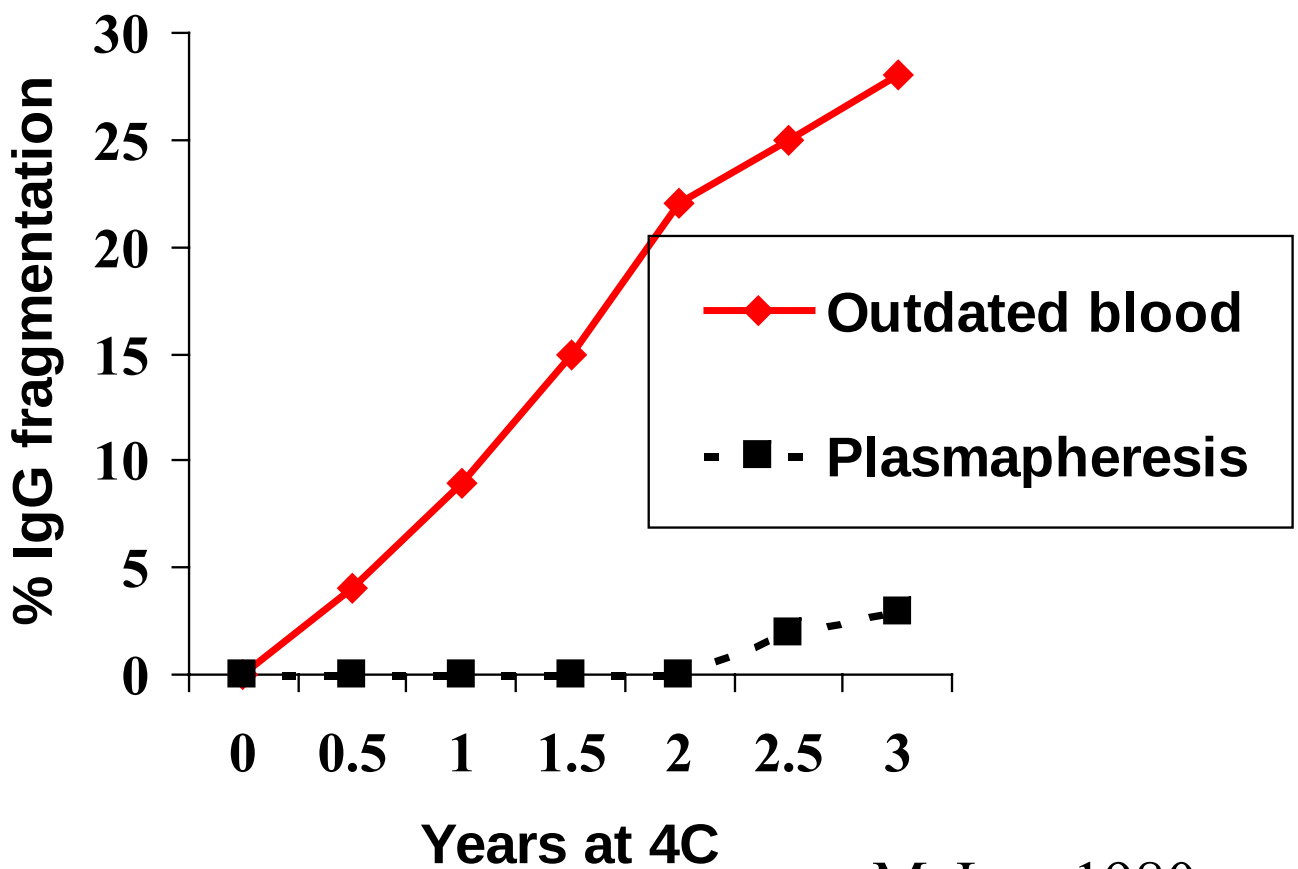
Prowse (1985) Dev Hem & Imm 13 :25-32

And of course, there are other things one can get out of plasma……..

Plasma Quality

Effect on IMIG fragmentation during storage

"……. *When obtained from whole blood, plasma intended solely for the recovery of proteins that are not labile in plasma is separated from cellular elements and frozen at –20 °C or below as soon as possible and at the latest within 72 h of collection..*" EP Monograph



McIver 1980

Plasma quality
Effect on albumin solutions

- Albumin made from plasma recovered from outdated blood shows higher PKA levels (BoB W/S 1977)
- Albumin made from haemolytic plasma was unstable at 25% concentration (Boros et al 1974)

……are these issues mainly of historical interest……or can other plasma proteins be affected by poor storage conditions?……

…..is this part of the great unknown…..and therefore subject to regulatory precautionism?…..

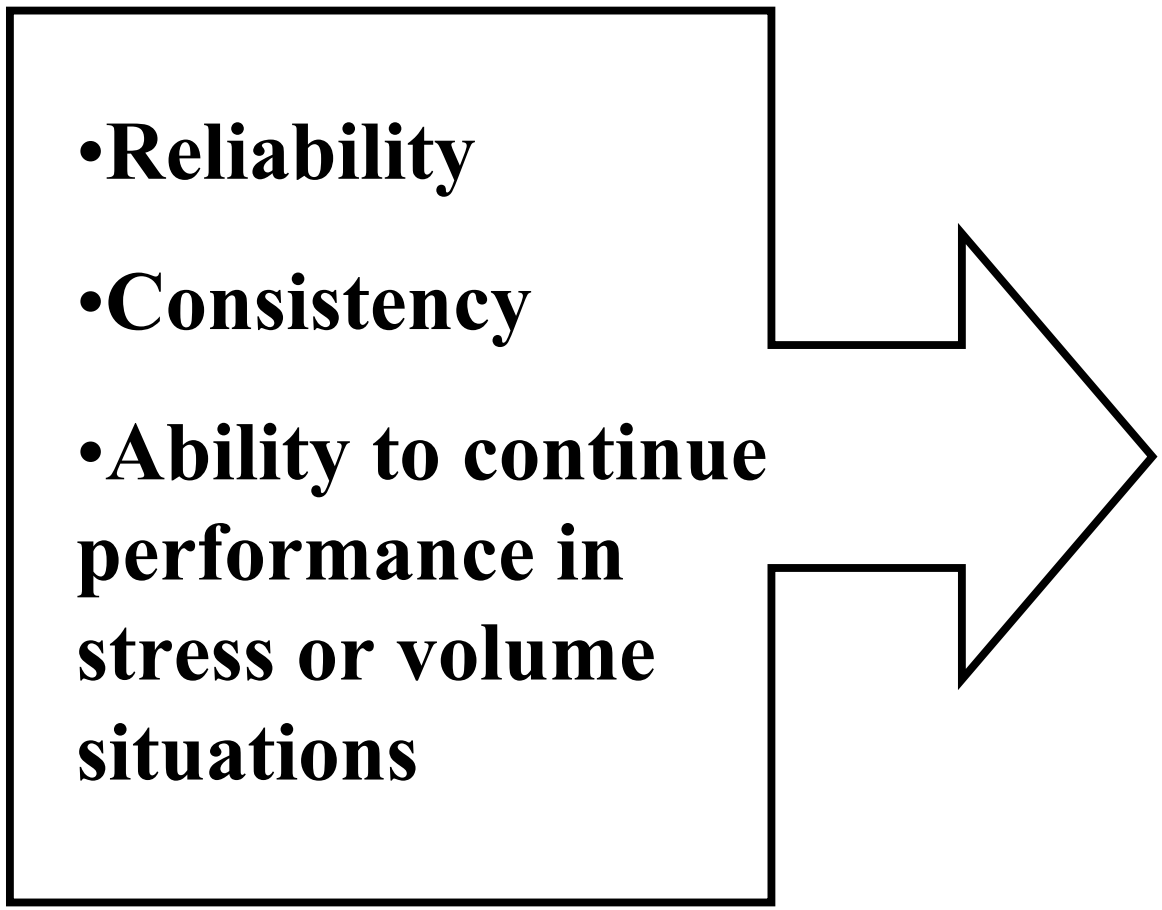
Plasma Manufacture
What is a quality product?

The characteristics of an item or process that indicate its conformance to designated parameters, and its degree of perceived customer acceptance or satisfaction. Quality characteristics often include ***reliability, consistency and the ability to continue performance in stress or volume situations,*** but are critical only in relation to the value placed on them by the user or customer.

http://www.bridgefieldgroup.com/glos7.htm

How can plasma be assured to a high level of ……..

- **Reliability**
- **Consistency**
- **Ability to continue performance in stress or volume situations**



⇒A defined manufacturing process

⇒Specified freezing and storage conditions

⇒Robustness to volume and temperature changes

Tentative conclusions and possible approaches

- There is a need for clear and unambiguous standards for plasma freezing and storage
- A process which results in a consistent product, irrespective of scale and location, should form the basis of any standard
- Empirical observations appear to support greater flexibility than some current requirements
- There is little evidence that any of these requirements have a bearing on product safety
- Basic conditions for minimising microbial contamination and preserving product integrity should be defined
- Other requirements reflecting product yield eg FVIII levels should be left to be negotiated between the manufacturer and plasma supplier

Thank you for reminding me of ……